Response to Referee #2

First of all, the authors thank the referee for submitting helpful and meaningful comments, which lead to improvements and clarifications within the manuscript.

Below, we provide our point-by-point responses. For clarity and easy visualization, the referee's comments (***RC***) are shown from here on in black. The authors' responses (***AR***) are in blue color below each of the referee's statement. In addition to the responses to referees' comments, we further modified the manuscript to increase its clarity and readability. The summary of other changes is included at the end of this document. We introduce the revised materials in green color along/below each one of your response (otherwise directed to the Track Changes version manuscript). All references are available in the end of this AR document.

RC: This paper is focused on the potential of domesticated animal feeding facilities as sources of atmospheric ice nucleating particles (INPs). Assessing anthropogenic sources of INPs is an important area of research. Hence, this is an area of research suitable for publication in ACP. However, there are some significant problems with this paper. Most importantly, the paper is not well written. These authors, including the first author, have produced some excellent pieces of work in the past so I know they are capable of much better. I do not wish to spend a great deal of time going from line to line trying to edit the manuscript for them. Instead I will focus on several key areas, which I'll work through here:
AR: The authors appreciate these general remarks and diplomatic criticisms regarding our manuscript by referee #2. We hope that with the changes made in the current version of the manuscript, the overall structure and readability improved such the quality of this paper (please read the Track Changes version paper). Further, a consistency of terms and symbols has been checked. Below, we provide our point-by-point responses in hopes of our manuscript being considered for another review by the reviewer.

RC: 1. 'Feedlot': it may be obvious what this is to a farmer in the USA, but it is not obvious what this is to the wider community. I had to google the term to find out. An alternative title could be 'Cattle feeding facilities in the USA are a sources of:
AR: This is a valid question. It is customary for authors to refer to "open-air feedlots" (OAFs), "animal-feeding operations" (AFOs) or even "concentrated [or confined] animal-feeding operations" (CAFOs), but those terms are not specific enough to distinguish the particular sort of production system we have in view as the referee mentioned. In the revised paper, for clarity, we have adopted the term "open-lot livestock facility" (OLLF) to denote a particular type of animal-feeding operation in which *livestock* (as distinct from poultry) is raised in *outdoor confinement* (as distinct from partially or totally enclosed housing, and also as distinct from pasture/range/"free-range" production systems). Open-lot livestock facilities are common in semi-arid and arid climates because, as contrasted with the alternative production systems typical of wetter and more temperate climates, they (a) are an intensified form of livestock production, generating more marketable product per unit land area with less built infrastructure, (b) make use of the elevated evaporative demand to reduce or eliminate precipitation-generated wastewater that must be controlled under water-quality regulations, and (c) capitalize on the nocturnal cooling characteristic of semi-arid and desert climates to avoid major investments in (and operating costs associated with) ventilation systems while still reducing the incidence and duration of livestock heat stress under most conditions. The authors now clarify our OLLF term in the third paragraph of the revised introduction as follows;

"Agricultural land use is in excess of 50% of total U.S. land use according to the U.S. Department of Agriculture, and there are > 26,000 "open-lot livestock facilities" (OLLFs) in the U.S. (Drouillard, 2018).

The term OLLF is adapted to denote a particular type of animal-feeding operation, in which cattle livestock is raised in outdoor confinement, as distinct from partially or totally enclosed housing, and also as distinct from pasture or free-range production systems (Auvermann et al., 2004). OLLFs are common in semi-arid and arid climates. Contrasted with the alternative production systems typical of wetter and more temperate climates, they (1) are an intensified form of livestock production, generating more marketable product per unit land area with less built infrastructure, (2) make use of the elevated evaporative demand to reduce or eliminate precipitation-generated wastewater that must be controlled under water-quality regulations, and (3) capitalize on the nocturnal cooling characteristic of semi-arid and desert climates to avoid major investments in (and operating costs associated with) ventilation systems while still reducing the incidence and duration of livestock heat stress under most conditions."

RC: 2. The abstract needs to be rewritten. Tell the reader about the conclusions of your work, not the topics you cover without an indication of what the key results and conclusions are. E.g.: 'New data on the ice nucleation (IN) properties of agricultural dust at heterogeneous freezing temperatures (Ts > -29_C) were generated, providing statistical context.'; 'Overall, we successfully characterized physical, chemical, and biological properties of aerosol particles found at a cattle feedlot'; 'The relationship between these measured properties and atmospheric IN parameterization relevant to mixed-phase clouds is discussed.' (This is deleted.); 'Our INP parameterization and ICR characterization are meaningful for improved understanding of INP emission and cloud microphysical processes in the supermicron-particle laden region'.
AR: We have re-written and re-formulated our abstract based on inputs from the reviewer.

RC: 3. Intro: these paragraphs are far too long and confusing. Break up into topics and build a logical case for this study.
AR**:** The logic of this section has been improved by introducing (1) the climatic impact of ice-nucleating particles (INPs), (2) previous fertile-and-agricultural soil dust-derived INP studies (Suski et al., 2018; Conen et al., 2011; Hill et al., 2016; Steinke et al., 2016; 2020; Tobo et al., 2014; O'Sullivan et al., 2014 – more discussion along with our results in Sect. 3.6), and (3) potential significance of soil dust INPs in the U.S. as well as Texas (and the reasons) in the first four paragraphs.

RC: 4. P2 ln 45. 'Milling and grinding'. It is inappropriate to mill natural samples of material where the larger sizes are likely made of different materials to the finer aerosolisable fraction. If, for example, the largest grains are ice active and they are milled down to sizes of atmospheric relevance then the ice nucleating ability you measure is likely to be simply a product of the milling process. Milled natural dusts like these samples are therefore not a meaningful proxy for the dust that may be aerosolised from this source, unless there is some justification for treating the sample in this way.
AR: This is a valid question, and the authors have a justification for this sample preparation procedure, which have been used for previous studies in this region. Physically pulverizing the manure samples by milling simulates in important ways the primary emissions mechanism at play in and characteristic of OLLFs. Although wind scouring is certainly one emissions mechanism that is occasionally responsible for fugitive aerosol emissions from OLLFs, by far the most significant emissions mechanism is the pulverization and airborne resuspension by animal hooves of the dry, uncompacted, friable manure that accumulates on pen surfaces in an OLLF in the southern High Plains and similar climates. In fact, the first known, bench-scale simulation of the emissions mechanism characteristic of OLLFs (Razote et al., 2006) featured an ultra-low-velocity "wind tunnel" with a test section in which both vertical and horizontal hoof action generated the aerosol. Further, a later evaluation tool designed for OLLF managers to conduct self-assessments of fugitive-dust potential (Bush et al., 2014; see Table 1, p. 818) is predicated on the greater

emissions potential associated with highly pulverized, nearly single-grained manure that develops on OLLF pen surfaces as manure accumulates, dries, and is crushed by animal hoof action, as contrasted with the small to medium-sized "clods" from which the single-grained material ultimately develops. Bush et al. (2014) also provide a more extensive account of fugitive-dust emission dynamics and their interaction with boundary-layer stability to create transient peaks in ground-level mass concentrations of fugitive dust. The short justification with citations is now provided in Sect. 2.2. as, "Physically pulverizing the surface samples simulates the primary emission mechanism and characteristic of OLLFs (Razote et al., 2006; Bush et al., 2014; von Holdt et al., 2021)."

The authors appreciate this question by the referee, and we cautiously note that there might be a bias between the field-sampled and lab-generated aerosol which might cause a difference in the ice nucleation ability (e.g. referencing Boose et al., 2016 c, who states "Furthermore, we find that under certain conditions milling can lead to a decrease in the ice nucleation ability of polymineral samples due to the different hardness and cleavage of individual mineral phases causing an increase of minerals with low ice nucleation ability in the atmospherically relevant size fraction."

RC: 5. Ln 46: Dry heat tests: what precedent is there for 100 C being a suitable test for deactivation of INP proteins? Ideally we would be shown control experiments with a biological ice nucleator.
AR: Ice nucleation activity by bacteria (Morris et al., 2004; Christner et al., 2008), fungi (Humphreys et al., 2001) and lichens (Henderson-Begg, et al., 2009) has been shown to be heat-sensitive irreversibly at 100 ^{o}C or below. This point is now addressed in Sect. 3.6 (please see the track changes manuscript).

RC: 6. Ln 48: Wet heat tests: Clarify the procedure here. The normal practice is to place a sealed vial in a volume of boiling water. The way the text reads is that the sample itself was boiled for 20 minutes. If this was the case, then how was the loss of water from the sample accounted for?
AR: What we did for our wet-heating is the normal practice that the referee mentioned. The falcon tube was closed and no water was lost. The sample tube was immersed in boiling water (~100 °C) for 20 minutes. This temperature was chosen to denature proteinaceous INPs. Thus, the subtraction of heated n_{INP} from non-heated n_{INP} might represent their contribution to immersion freezing. This procedure is adapted from Schiebel (2019). Briefly, the aerosol particle suspension (3 mL) from a non-treated stock was first transferred to a sterile 50ml falcon tube. The screw-cap is closed, such that no water is lost. Then the tube is placed together with a precisely fitting styrofoam ring in a water-filled glass beaker. The styrofoam ring ensures that the tube is floating and that all of the aerosol suspension is below the water surface for best heat transfer. The beaker is covered with aluminum foil and is placed on a stirring hot plate to boil the water. The sample tube remains in the boiling water for 20 min.

RC: 7. P7. Section 3.3/ The first two paragraphs have little to do with the heading of this section.
AR: The authors agree. Moved to Sect. 2.4.

RC: 8. Section 3.4 and Table 5. Why is there a parameterisation for each sample? This seems excessive. It would be more useful to have a single parameterisation with an indication of variability.
AR: Offering a universal or representative single parameterization for agricultural INPs is not the scope of this work. As OLLF represents a point source of fresh livestock-generated dust, we expect that it would have different ice nucleation efficiency than aged/weathered dusts. Individual parameterizations are useful to analyze spectra by comparing $\Delta\log(n_{s,geo})/\Delta T$ values etc. We would like to keep all individual parameterizations. As now seen in Sect. 3.5, we used the Field_Median parameterization as an example (and representative) one for its T coverage up to -5 °C. The summary table of all parameterizations and associated text are now moved to SI Sect. S6 to increase the readability of the main manuscript and to

focus on the main scientific discussion on verifying that OLLF can be an ecosystem acting as a source of soil dust INPs. This point is now clarified in SI Sect. S6 (please see the track changes version).

RC: 9. P7, ln 34. 'this parameterization can be easily incorporated in many model platforms'. The authors need to be more specific how they envisage that this might happen. Do relevant models have this source of dust in them already with the emission already set up as an independent tracer? I suspect the answer is no. So, what else would we need to know in order to be able to represent this source of INP?
AR: The reviewer is right. We admit that it was not written in a right tone. As this statement is too ambitious, we removed this sentence. Besides, our main objective of this study is to verify that OLLF can be an ecosystem acting as a source of soil dust INPs but not providing a computationally unexpansive universal soil dust IN parameterization. For these reasons, we decided to omit this paragraph completely from the manuscript. In general, incorporating IN in any cloud/atmospheric models is a complex effort of dealing emission flux, aerosol dynamics, cloud microphysics, IN parameterization, and cloud macrophysics etc. (Zhang et al., 2018). Thereby, we understand that it is not reasonable to use the word like "easily" etc.

RC: 10. Ln 46. ' no notable difference after dry-heating was observed for both TXD01 and TXD05, representing an important negative result'. Why is this important? Is it significant that there is no effect of heating the sample dry to 100 C. It would only be significant if IN proteins are known to deactivate on heating to this temperature.
AR: This negative result agrees with our metagenomics analysis, where no known ice nucleation active bacteria were detected. We clarified our point by rephrasing this sentence to, "Thus, no notable difference after dry-heating was observed for both TXD01 and TXD05 (**Table 5**). This negative result is important because it agrees with our metagenomics analysis, where no known IN-active bacteria were detected."

RC: 11. P 8 section 3.7: State which ns parameterisation was used in this calculation
AR: "Field_Median" as stated in the text. We now state, "Due to the atmospheric relevance and T coverage extending to -5 °C, we used a fit of ***Field_Median*** in **Table S3** to compute representative $n_{s,geo}$ relevant to OLLF."

RC: 12. P 8. Section 3.7: Equation 1 is only appropriate if a small fraction of INP at any one size is activated to ice. This equation does not take into account the number of dust particles. For example, if there were 10 dust particles per cm3, this parameterization might predict 1000 dust cm-3, which would clearly be nonsense. To predict INP concentrations using an ns (or similar) parameterisation, you either need to be able to prove that only a small fraction of particles at one size over the whole size range activate to ice or integrate over the size distribution.
AR: The reviewer is right. Niemand et al. (2012) infers that the usage of n_s is valid for small percentages of IN active fraction (~1%). From the numbers of $N_{total,0}$ given in our Table 3 (total number concentration of particles at the initial stage prior to expansion), we know we examined on average ~ 200,000 L^{-1} of aerosol particles in the immersion freezing mode in ADIA. Even assuming we evaluate INP up to 2,000 L^{-1}, our INP fraction is 1%. Thus, our n_s parameterization is reasonable. Now, this point is clarified in Sect. 2.6 (please see the track changes version).

RC: 13. P8. Section 3.7: Why use ns. The data seems to spread out in ns substantially, but in nm, they collapse. Hence, nm seems to be a better way of doing this calculation.
AR: We have presented a comparison of our n_s results to six different n_s parameterizations from previous (yet recent) soil dust IN studies to relatively assess the IN efficiency of OLLF dust to them. Such a rigorous comparison is invaluable, and we would like to keep using n_s instead of n_m. If we were to analyze ice nucleation active macromolecules (INM) (i.e., the biological protein complexes), the number of INM

scaled with the known amount of mass of specimen in a droplet would make sense as IN is triggered by biological component, which may have a mass of ~ 150 kDa, rather than on insoluble surface (Wex et al., 2015 and references therein). However, as we did not find any notable amount of known IN active microbiome in our OLLF samples, we believe that our usage of n_s is valid. Therefore, in the study presented here, the ice nucleation ability will be expressed per unit surface of OLLF sample/particle.

RC: 14. Ln 50. 'which is three orders of magnitude higher than typical ambient INP concentration from continental sources'. This is a selective reading of the literature. The values are certainly high, but they are not 1000 times higher than recent literature values. For example, other studies also report high INP concs: Petters and Wright (2015) show values up to 1000 L-1 and O'Sullivan et al. (2018) report values approaching 100 L-1 and Suski et al. (2018) report values in excess of 100 L-1.
AR: Now, the revised manuscript discuss this point in Sect. 3.6. Comparison to previous soil dust IN studies. Please see our revised manuscript. We also provide a summary of how high OLLF INPs could be as compared to other solid dust INPs in Fig. 10.

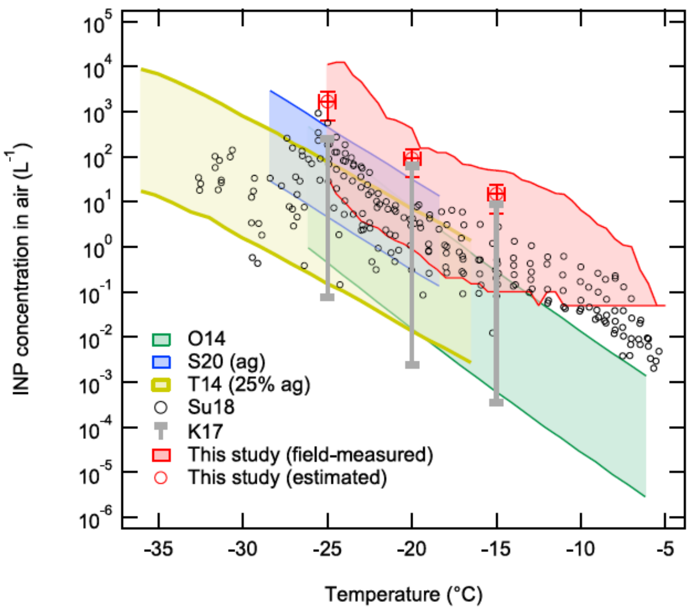

Figure 10. Ambient INP concentrations of soil dusts and aerosol particles as a function of T. The red-shaded area represents the range of our field n_{INP} values at 0.5 °C interval for -5 °C > T > -25 °C from this study (Fig. 4). The red open symbols are our estimated median (± standard deviation) at -15, -20, and -25 °C discussed in Sect. 3.5. Five reference data are adapted from O'Sullivan et al. (2014 Fig. 9; O14), Steinke et al. (2020 Fig. 3; S20), Tobo et al. (2014 Fig. 6b; T14), Suski et al. (2018 Fig. 1a-d; Su18), and Kanji et al. (2017 Fig. 1-10; K17). Note that we display the maximum and minimum at -15, -20, and -25 °C of K17 in comparison to our estimation.

RC: 15. P 9 ln 4. What are 'controlled-experiments'?
AR: We meant to say the temperature-controlled laboratory experiments. We now revised our conclusion as suggested by referee #2, and this sentence is removed.

RC: 16. Conclusions: I found this hard to follow. There is a lack of structure and several statements do not seem to follow on logically. E.g.: In ' The insignificance of dry heating was demonstrated with the increase of organics found for the ICR of dry-heated samples' the second statement does not follow on from the first.

AR: This section has been revised. We have rephrased the sentence that the referee pointed out to; "Even after dry heating treatment, the increase of organics fraction was found for the ICR of our OLLF samples."

Other revisions:

West Texas → the Texas Panhandle
The authors recently learned that ACP does not accept "West Texas" as a proper noun. As the place name needs to have clearly defined boundaries and be internationally known, we decided to adapt "the Texas Panhandle" and replaced it with all West Texas in this paper. At its first appearance in the manuscript, we define it as "the Texas Panhandle (northern most counties of Texas; also known as West Texas)".

Dr. Larissa Lacher has been added as a coauthor because she has visited the Texas Panhandle to in part support the field sampling activities at OLLFs and was involved in analyzing some INSEKT samples, which led to improve SI Sect. S3.

We have a new acknowledgements section and separated financial support statements.

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